

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 1-36 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter that the applicant regards as the invention. Specifically, it is the Examiner's view that it is not clear what subject matter is encompassed by the recitation of the term "dairy source."

Applicants respectfully submit that the present invention encompasses both *in vivo* and *in vitro* dairy sources, as set forth in detail in the as-filed specification. Specifically, the specification contains numerous examples of "dairy sources" being both an isolated dairy source (such as cheese processing waste streams) and an *in vivo* dairy source (such as the use of transgenic animals to produce higher than normal levels of sialyloligosaccharides before isolation of milk from the animals).

For example, the definition of "dairy source" at lines 1-5 on page 11 of the specification includes "[A] product of lactation in a mammal, a substance made by the product, or a byproduct thereof." This embodiment of the present invention encompasses isolated dairy sources.

By way of another example, the paragraph from line 23, page 9 to line 6, page 10 of the specification sets forth that the present invention provides a method for enriching $\alpha(2,3)$ -sialyllactose in milk "[U]sing transgenic mammals that express an $\alpha(2,3)$ -trans-sialidase transgene." The milk produced by such transgenic animals of this embodiment of the present invention contains enriched $\alpha(2,3)$ -sialyllactose concentrations. Accordingly, the milk of such transgenic animals is an *in vivo* dairy source. Further support for *in vivo* dairy sources of the present invention can be found in the specification from line 31, page 56, through line 21, page 57. Applicants respectfully submit that the rejection under 35 U.S.C. § 112, second paragraph, is improper and should be withdrawn.

The Examiner has also rejected claims 1-36 as incomplete, and therefore indefinite, as allegedly being unclear with respect to how trans-sialidase relates to the production of sialyloligosaccharides in the present invention. While not acquiescing to the Examiner's rejection, but in an interest to expedite prosecution of the instant

application, Applicants have amended independent claims 1 and 19 to recite the method step, "...whereby at least one sialyloligosaccharide is produced in said dairy source." Applicants have also amended independent claim 36 to recite a similar method step, namely, "...whereby α -2,3-sialyllactose is produced in said dairy source."

Applicants submit that, as amended, claims 1-36 (independent claims 1, 19 and 36, and therefore also dependent claims 2-18 and 20-35) are not incomplete, and therefore not indefinite. Specifically, the amended claims more clearly describe the nexus between the trans-sialidase and the sialyloligosaccharides of the present invention, because the invention encompasses the production of sialyloligosaccharides as a direct result of the methods of the invention. Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

The Examiner also rejects claims 1-36 on the ground that many dairy products "already contain" sialyloligosaccharides, and as such, the claims are confusing, because the starting material contains the desired product. Applicants respectfully submit that the point of the present invention is to **produce** an amount of sialyloligosaccharides in a dairy source, and as such, the starting material may or may not contain sialyloligosaccharides. In fact, in the Summary of the Invention, at lines 20-26 on page 7 of the present application, Applicants expressly state, "The methods of the invention have particular applications in **producing** α (2-3) sialyllactose in a dairy source prior to, during, or after processing of the dairy source during the cheese manufacturing process, thereby greatly **increasing the recoverable yield of α (2-3) sialyllactose** from the dairy source." (Emphasis added).

In the specification at lines 10-11, Applicants state that a possible dairy source of the invention can be "a composition simulating milk." At lines 21-26 on page 30, Applicants set forth that "a composition simulating milk is a solution containing at least α (2-3) sialosides to act as **donors** for the α (2,3) trans-sialidase and lactose, and wherein the presence of free sialic acid, CMP-sialyltransferase and/or CMP-synthetase is not required to drive the sialylation of lactose by α (2,3) trans-sialidase." (Emphasis added). In this one particular aspect of the invention, Applicants demonstrate the requirement of a carbohydrate-sialic acid adduct as part of the invention.

Further, in the specification at lines 8-16 on page 14, Applicants set forth that "Starting materials may therefore be derived from...a mixture of lactose and $\alpha(2-3)$ **sialylated saccharide compositions**" which simulate a dairy source, providing that the starting material may contain sialyloligosaccharides (Emphasis added). Further still, Table 1, on page 64 of the specification, demonstrates a measurable presence of $\alpha(2-3)$ sialyllactose in a dairy source before effective use of the present invention to increase the amount of $\alpha(2-3)$ sialyllactose in that dairy source.

Applicants respectfully submit that the present invention is indeed directed to producing (i.e., "increasing" the amount of) sialyloligosaccharides in a dairy source, regardless of whether the starting material contained **any** sialyloligosaccharides. Accordingly, Applicants submit that the rejection under 35 U.S.C. § 112, second paragraph, is improper and should be withdrawn.

Claim 11 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the term "lactose," as it is allegedly unclear as to how lactose differs from any of the other lactoses recited in the claim. Applicants respectfully submit that one of skill in the art would undoubtedly know and understand the difference between various forms of lactose. However, in an effort to expedite prosecution of the instant application, Applicants have amended claim 11 to recite, "...crystallized lactose, spray dried lactose, whey powder, edible lactose and **aqueous** lactose." Applicants submit that claim 11, as amended, is not unclear with respect to the term "lactose." Accordingly, Applicants respectfully request withdrawal of the rejection of the term "lactose" under 35 U.S.C. § 112, second paragraph.

Support for the term "aqueous lactose" can be found in the as-filed specification at lines 16-27 on page 48. Specifically, the two paragraphs at lines 16-27 on page 48 show the utility of an aqueous lactose solution with respect to the present invention. Further, lines 16-17 describe the relationship between aqueous lactose and crystalline lactose in a lactose mother liquor. The above-referenced description of aqueous lactose demonstrates that an aqueous form of lactose is a general form of lactose with respect to the present invention, and it describes lactose that has not necessarily been subjected to further processing (such as crystallization) or combined with other components to form a heterogeneous mixture (such as whey permeate). Further still,

lines 21-36 on page 35 of the specification set forth various forms of dairy processing waste streams, including "lactose," as well as the aqueous, lactose-containing mother liquor resulting from crystallization of lactose.

Rejections under 35 U.S.C. § 112, first paragraph – enablement

Claims 1-35 have been rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the as-filed specification. Specifically, it is the Examiner's view that the specification does not reasonably provide enablement for the preparation of any and all sialyloligosaccharides from any and all dairy products. Applicants respectfully disagree and submit that the claims are enabled for the reasons set forth below.

Claim 1, as amended, recites a method of producing sialyloligosaccharides in a dairy source, using an $\alpha(2-3)$ trans-sialidase. As set forth in the specification at lines 7-14 on page 10, a "trans-sialidase" is an enzyme that catalyzes the transfer of a sialic acid from one saccharide-containing molecule to another saccharide-containing molecule. As known to one of skill in the art, an enzyme, such as a glycosidase or a glycosyltransferase, is named for the specificity of the reaction that the enzyme catalyzes.

Therefore, the $\alpha(2-3)$ trans-sialidase is named as such because this particular enzyme has the ability to transfer a sialic acid residue that is $\alpha(2-3)$ -linked to a saccharide donor moiety from that donor moiety to a saccharide acceptor moiety. Further, $\alpha(2-6)$, $\alpha(2-8)$, and $\alpha(2-9)$ terminal sialyl residues cannot serve as donors for $\alpha(2-3)$ trans-sialidase. See, e.g., Vandekerckhove et al. (Glycobiology, 1992, 2(6):541-548) and Colli (FASEB J., 1993, 7:1257-1264), cited in the Information Disclosure Statement submitted with the instant application at the time of filing.

Further, the acceptor specificity of the $\alpha(2-3)$ trans-sialidase of the present invention was also well known at the time of filing of the present application. Colli describes the acceptor specificity of the *T. cruzi* $\alpha(2-3)$ trans-sialidase of the instant invention as being directed to an oligosaccharide having a β -linked terminal galactose moiety. Colli further describes that oligosaccharides having β -linked terminal N-acetyl-galactosamine, glucose, or N-acetyl-glucosamine are **not** acceptors for $\alpha(2-3)$ trans-sialidase. Further, oligosaccharides having α -linked terminal galactose or glucose

moieties are not acceptors for $\alpha(2-3)$ trans-sialidase. Further yet, Colli sets forth that internal β -linked galactose moieties are not acceptors for $\alpha(2-3)$ trans-sialidase, and that the mere presence of a fucose moiety near a β -linked terminal galactose moiety will diminish the capability of such an oligosaccharide to serve as an acceptor for $\alpha(2-3)$ trans-sialidase.

Applicants respectfully submit that, based on the very specific donor and acceptor requirements for the $\alpha(2-3)$ trans-sialidase claimed in the present invention, one of skill in the art would understand and expect that a sialyloligosaccharide product of the present invention would encompass any sialyloligosaccharide capable of being produced using an $\alpha(2-3)$ trans-sialidase. More specifically, one of skill in the art would understand the donor and acceptor specificities of the claimed $\alpha(2-3)$ trans-sialidase and would therefore expect that a sialyloligosaccharide product of the present invention would most likely contain a terminal sequence of -galactose-sialic acid, wherein sialic acid is the terminal residue of the sialyloligosaccharide product obtained using a method of the present invention. It would then require no more than routine and ordinary experimentation for one of skill in the art to ascertain the nature and amount of sialylated oligosaccharides obtained using a method of the present invention, as set forth more fully below.

An Example in section 5.7 in the specification, pages 61-64, in toto, describes in detail the production of $\alpha(2,3)$ sialyllactose in milk. Lines 14-27 on page 61 of the specification describe the milk used as the dairy source, the specific number of units of trans-sialidase enzyme used for the production of $\alpha(2,3)$ sialyllactose in the milk, and the specific reaction conditions needed to effect the production of $\alpha(2,3)$ sialyllactose in the milk. Further, the table set forth at lines 8-27 on page 64 of the specification describes, in detail, the amount of $\alpha(2,3)$ sialyllactose produced in the milk dairy source as a result of the reaction conducted according to the specific conditions as set forth above.

A second Example is set forth in section 5.8 at lines 1-28 on page 65 of the specification. This Example describes the enrichment of $\alpha(2,3)$ sialyllactose in various dairy sources and waste streams, as illustrated in Figure 5 of the as-filed

application. Further, Applicants describe elsewhere throughout the specification the nature of various dairy sources of the invention, as well as the basis of the utility of various dairy sources of the invention. Specifically, Applicants set forth at lines 27-30 on page 7 of the specification that "dairy sources and cheese processing waste streams" are known to contain high concentrations of lactose, an oligosaccharide terminating in a β -galactose moiety, and numerous $\alpha(2,3)$ sialosides, which are known to be specific donors for $\alpha(2,3)$ trans-sialidase. Accordingly, one of ordinary skill in the art would undoubtedly look to any dairy source, including a cheese processing waste stream, in order to practice the instant invention. Further, one of ordinary skill in the art would reasonably expect that any oligosaccharide terminating in a β -galactose moiety and present in a dairy source is a potential acceptor for $\alpha(2,3)$ trans-sialidase activity in that dairy source. Differential analysis of oligosaccharides terminating in sialic acid in a dairy source treated with $\alpha(2,3)$ trans-sialidase according to the present invention and in a dairy source not treated with $\alpha(2,3)$ trans-sialidase would readily identify products of the methods of the present invention, and would not require undue experimentation.

Applicants respectfully submit that, based on the above-described Examples, one of skill in the art would have ample guidance in successfully carrying out the present invention. Further, the multitude of Examples and data provided in the instant application would provide sufficient guidance in practicing the present invention using a dairy source. Based on the reaction conditions provided by Applicants in the specification, including the various suitable dairy sources, the range of amounts of trans-sialidase required to effect catalytic activity, the range of suitable reaction conditions, the pH range possibilities for the reaction mixture, the range of usable temperatures for the reaction mixture, the range of length of time the reaction must be conducted, and multiple methods of recovering and analyzing the $\alpha(2,3)$ sialyllactose produced in the dairy source, Applicants submit again that one of skill in the art would not need to employ undue experimentation to practice the present invention.

Further, the two above-mentioned specific Examples illustrating a reduction to practice of the present invention provide ample guidance to the skilled artisan, further limiting the amount routine and ordinary experimentation needed to

practice the present invention.

The test for undue experimentation requires that several factors be considered. These factors include:

- (A) The breadth of the claims;
- (B) The nature of the invention;
- (C) The state of the prior art;
- (D) The level of one of ordinary skill;
- (E) The level of predictability in the art;
- (F) The amount of direction provided by the inventor;
- (G) The existence of working examples; and
- (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

(MPEP 2164.01(a)). Regarding the instant application, Applicants respectfully submit that the facts, data, and arguments set forth above demonstrate that the specification provides ample guidance to one that desires to practice the invention, that the field of endeavor of the present invention is predictable and well-established, that numerous working examples have been provided in the specification, that the level of skill in the relevant art is high, and that the breadth of claims 1-36 is not unduly broad. Accordingly, the present invention would not require undue, but at most, only routine experimentation by one who desires to practice the instant invention. Accordingly, Applicants respectfully submit that the rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement is improper and should be withdrawn.

Rejection under 35 U.S.C. § 102(b)

Claim 36 was rejected under 35 U.S.C. § 102(b) as being anticipated by Vandekerckhove et al. (1992, Glycobiology 2:541-548). Specifically, it is the Examiner's view that Vandekerckhove et al. discloses the preparation of 2,3-sialyllactose by contacting lactose with 2,3-trans-sialidase in the presence of various sialyloligosaccharides.

While not acquiescing to the Examiner's rejection, but in an attempt to expedite prosecution of the present application, Applicants have amended claim 36 to recite, "...A method of producing 2,3-sialyllactose in a dairy source..."

In order for a rejection under 35 U.S.C. § 102(b) to be proper, each and

every element of the invention must be disclosed by the cited reference. The Vandekerckhove reference does not disclose a dairy source in the production of 2,3-sialyllactose. Therefore, the Vandekerckhove reference does not disclose each and every element of claim 36. Accordingly, Applicants respectfully submit that claim 36, as amended, is not anticipated by Vandekerckhove, and that the rejection under 35 U.S.C. § 102(b) has been overcome.

Rejection under 35 U.S.C. § 103(a)

Claims 1-35 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Vandekerckhove et al. in view of Brian et al. (U.S. Patent No. 5,575,916, issued November 19, 1996; the "916 patent") and Ito et al. (U.S. Patent No. 5,409,817, issued April 25, 1995; the "817 patent"). Specifically, it is the Examiner's view that it would have been obvious for one of skill in the art to practice the present invention when considering Brian's disclosure of the presence of 2,3-trans-sialidase substrates in dairy products and Ito's disclosure of the utility of 2,3-sialyllactose produced by enzymatic action on sialyl Lewis x, in light of the disclosure of Vandekerckhove (described above in response to the 35 U.S.C. § 102(b) rejection). Applicants respectfully submit that claims 1-35 are not obvious for the reasons set forth below.

In order for the rejection under 35 U.S.C. § 103(a) to be proper, the Examiner establish a *prima facie* case of obviousness. More specifically, it must be shown that there is some suggestion or motivation to combine the cited references, that the prior art provides one of ordinary skill in the art with a reasonable expectation of success, and that the combination of the art teaches or suggests each and every element of the rejected claims.

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in

the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

Applicants submit that the Ito patent teaches that the *T. cruzi* α -2,3,-trans-sialidase is **not useful** in synthesis reactions, **due to the reversible nature of the catalyzed reaction**. In fact, Ito enzymatically couples the 2,3-trans-sialidase reaction to another enzymatic reaction in order to shift the equilibrium and favor the 2,3-trans-sialidase activity. The present invention discloses that α -2,3-sialyltransferase can be used **alone** to produce α -2,3-sialyllactose in a dairy source. Accordingly, Ito **teaches away** from the present invention, and as such, Applicants respectfully submit that the rejection under 35 U.S.C. § 103(a) is improper.

Because there is no suggestion or teaching in the cited art regarding the use of an α -2,3-sialyltransferase **alone** in order to produce α -2,3-sialyllactose in a dairy source, the combination of Vandekerckhove, Brian and Ito does not teach or suggest all of the claim limitations of the present invention. Further, because one of skill in the art would not have been motivated to use an α -2,3-sialyltransferase **alone** in order to produce α -2,3-sialyllactose in a dairy source, one of skill in the art would not have had a reasonable expectation of success in arriving at the present invention. Accordingly, Applicants respectfully submit that rejection under 35 U.S.C. § 103(a) is improper and request withdrawal of the rejection.

Double Patenting Rejection

The Examiner has rejected claims 1-36 under the judicially created doctrine of obviousness-type double patenting. Specifically, it is the Examiner's view that the above-mentioned claims are not patentably distinct from claims of U.S. Patent No. 6,323,008 of Pelletier et al. ("the '008 patent").

Applicants understand that a timely filed Terminal Disclaimer in

compliance with 37 CFR § 1.321(c) may be used to overcome such a non-statutory type of double patenting rejection. Accordingly, Applicants are filing the appropriate Terminal Disclaimer herewith. Therefore, Applicants respectfully submit that the Double Patenting rejection has been overcome.

Summary

The amendments made herein are supported in the as-filed specification, and as such, no new matter has been added by way of the present amendment. Applicants respectfully submit that each and every rejection or objection set forth by the Examiner has either been overcome or is now inapplicable, and that the instant application is in full condition for allowance. Favorable examination of the claims on the merits is respectfully requested.

Respectfully submitted,
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(Date)

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Marked-up version of the claims to show changes made

Please amend claims 1, 11, 19 and 36 without prejudice, as set forth below.

1. (Amended) A method for producing sialyloligosaccharides in a dairy source comprising:

(i) contacting a catalytic amount of at least one $\alpha(2-3)$ trans-sialidase with a dairy source to form a dairy/trans-sialidase mixture; and

(ii) incubating said dairy/trans-sialidase mixture under conditions suitable for $\alpha(2-3)$ trans-sialidase activity;

whereby at least one sialyloligosaccharide is produced in said dairy source.

11. (Amended) The method of claim 3 wherein the cheese processing waste stream comprises a member selected from the group consisting of: whole whey, demineralized whey permeate, a regeneration stream from demineralized whey permeate, whey permeate, crystallized lactose, spray dried lactose, whey powder, edible lactose and aqueous lactose.

19. (Amended) A method for producing sialyloligosaccharides in a cheese processing waste stream comprising:

(i) contacting a catalytic amount of at least one $\alpha(2-3)$ trans-sialidase with a cheese processing waste stream to form a waste stream/trans-sialidase mixture; and

(ii) incubating said waste stream/trans-sialidase mixture under conditions suitable for $\alpha(2-3)$ trans-sialidase activity;

whereby at least one sialyloligosaccharide is produced in said dairy source.

36. (Amended) A method for producing α -2,3-sialyllactose in a dairy source comprising:

(i) contacting a catalytic amount of at least one α -2,3-trans-sialidase with lactose and an α -2,3-sialyloligosaccharide, in the absence of CMP-sialyltransferase, to form a mixture; and

(ii) incubating said mixture under conditions suitable for α -2,3-trans-sialidase activity;

whereby α -2,3-sialyllactose is produced in said dairy source.